OXITEC

October 11, 2019

OPP Docket
Environmental Protection Agency Docket Center
1200 Pennsylvania Ave, NW
Washington, DC 20460

Re: Docket ID No. EPA-HQ-OPP-2019-0274. Pesticide Experimental Use Permit; Receipt of Application; Comment Request (93167-EUP-E)

Dear Sir or Madam,

Oxitec appreciates the opportunity to provide these comments in response to the US Environmental Protection Agency's (EPA) request following receipt of Oxitec application 9316-EUP-E. In particular, we would like to provide additional context, information and technical details necessary to address a few salient topics that have been previously raised. Oxitec has submitted a rigorous and comprehensive data package evaluating our OX5034 technology to demonstrate that deployment of OX5034 mosquitoes will not cause unreasonable adverse effects to human health and the environment following release under the submitted experimental use permit (EUP). We look forward to EPA's review and evaluation of our dossier.

Background: Technical Overview and History of Successful Deployments

Oxitec's 1st generation self-limiting mosquito technology (OX513A), successfully deployed in multiple locations including in Brazil, the Cayman Islands and Panama, has been succeeded by the new 2nd generation self-limiting mosquito, OX5034. The OX5034 mosquito carries many of the key features of OX513A that made it a safe, effective control method for reducing Aedes aegypti mosquito populations. These include effective mosquito control, non-toxic and non-allergenic active and inert ingredients, a lack of direct effects on non-targeted species, and no long-term effects or chemical residues in the environment. OX5034 also has several additional features, including genetic sex-separation, which enables more cost-effective production and release of only male mosquitoes, and a brighter fluorescent marker, which enables field monitoring in all life stages of the mosquito.

The 2nd Generation *Aedes aegypti* carries a self-limiting gene that prevents female offspring from surviving, allowing for male-only production and deployment. After releases of OX5034 males into the field, which find and mate with wild female mosquitoes, reduction of the target population is achieved as the female offspring of these encounters cannot survive. Male progeny survive, carrying a copy of the self-limiting gene; in turn, these males are able to pass on the self-limiting gene to half of their offspring, of which female carriers of the gene cannot survive. The self-limiting gene can thereby persist but declines over time, offering potentially multiple but still self-limiting generations of suppression for every Oxitec OX5034 *Aedes aegypti* male released.

The 2nd generation mosquito has been successfully tested in Brazil. In partnership with the municipal vector control authorities in the city of Indaiatuba, the pilot project demonstrated the new strain's effectiveness in suppressing populations of the *Aedes aegypti* mosquito – the primary vector of dengue, Zika, chikungunya and yellow fever – in four densely populated urban communities across the city. Post-trial monitoring has also confirmed that the self-limiting gene does indeed decline and disappear post-release.

Releases of Oxitec's OX5034 *Aedes aegypti* were conducted in four separate communities under approval issued by Brazil's national biosafety authority, CTNBio, during a year-long trial starting in May 2018. The trial was designed to test a number of performance features of the 2nd Generation OX5034 *Aedes aegypti*, including the performance outcomes generated by the use of two different mosquito release rate levels in dense urban environments. Abundance of wild *Aedes aegypti* was monitored before and during the release program to allow for an accurate evaluation of the trial's impact. Wild *Aedes aegypti* numbers were kept at low levels throughout the high season in all treated neighbourhoods, whereas populations in areas untreated by Oxitec's OX5034 *Aedes aegypti* rose as normal.

Relative to the untreated control area, releases of OX5034 male mosquitoes achieved an average of 89% peak suppression across two communities treated with a low release rate of mosquitoes and an average of 93% across two communities treated with a higher release rate. The optimal suppression observed was in one community wherein a 96% peak suppression with the high release rate over a four-week period was achieved. ("Peak suppression" is measured using the highest sustained suppression over a four-week period in an Oxitec-treated site when compared to a control site untreated by Oxitec mosquitoes for the same period of time. This measures the intervention's sustained suppression effect over time, which is a more accurate measure than selecting suppression results from a single day or week.)

Addressing Key Issues:

Oxitec would like to proactively address and respond to a few key issues/claims that have also been highlighted in comments placed in the docket. These issues can best be categorized as follows:

- Scientific questions and potential implications raised following release of a recently published paper titled "*Transgenic Aedes aegypti Mosquitoes Transfer Genes into a Natural Population*" (Evans et al., 2019, *Scientific Reports*, Vol 9, Article number: 13047);
- Is Oxitec's OX5034 self limiting mosquito a gene drive technology?; and
- Will the use of tetracycline antibiotics in the production of Oxitec mosquitoes lead to antibiotic resistance?

Assessment of Scientific Reports Article "Transgenic Aedes aegypti Mosquitoes Transfer Genes into a Natural Population" (Evans et al. 2019 Scientific Reports, Vol 9, Article number: 13047).

In response to the recently published open access paper titled "*Transgenic Aedes aegypti Mosquitoes Transfer Genes into a Natural Population*" (Evans et al. 2019 *Scientific Reports*, Vol 9, Article number: 13047), provided here are responses to address the range of misleading, unsubstantiated and speculative statements made in the paper about Oxitec's OX513A mosquito technology.

Fact Checks:

- There was no negative, deleterious or unanticipated effect to people or the environment from the release of OX513A mosquitoes documented by this study;
- The paper reports that OX513A releases successfully reduced the wild mosquito population, as the mosquito was designed to do.
- This paper's data is entirely consistent with the large body of historical peer-reviewed scientific literature relating to OX513A and Oxitec's technology more broadly;
- As indicated in published data (Garziera et al., 2017), the OX513A self-limiting gene does not persist in the environment. Monitoring around all of Oxitec's studies has documented complete disappearance of the inserted genes from the environment;
- Natural genes carried by Oxitec mosquitoes do not confer increased capacity to transmit disease nor resistance to commonly used insecticides;
- The natural genes passed on by the few surviving OX513A mosquitoes died out in treated areas after releases stopped;
- Oxitec has a robust monitoring and quality control system approved by regulators wherever releases occur and has detected no abnormalities or negative consequences relating to the presence of Oxitec's mosquitoes, their self-limiting genes, or OX513A's natural background genetics;
- *Aedes aegypti* is an invasive, non-native mosquito species in Brazil and throughout most of the world, and thus the natural background genetics of Oxitec's strain, along with the wild *Aedes aegypti* found locally in Brazil, are both introduced to the area.

The speculations made in the paper are not supported by any data in the paper or in any of the historical peer-reviewed data or studies. Each of the key facts is supported by independent peer-reviewed studies, publications, and Oxitec's regulatory findings.

Response to Paper's Speculative Statements:

Several of the statements in the paper represent speculation and are not supported by scientific data – either in the paper itself or in the larger body of peer-reviewed literature on Oxitec's technology. These include:

- The hypothesis that introducing background genetics would lead to increased "hybrid vigor" in the mosquito. ***The data published in this paper and in the entire body of peer-reviewed literature do not support this hypothesis***.
- The hypothesis regarding introgression [passing on of genes other than the self-limiting gene from OX513A, into the wild mosquitoes], suggesting that this may introduce other relevant genes such as with insecticide resistance. ***To the contrary, Oxitec has demonstrated that OX513A is not resistant to commonly used insecticides*** (Carvalho et al., 2015).

- The hypothesis regarding the potential impact of introgression on disease control and transmission. The data in the paper itself demonstrate that OX513A is not a more competent disease vector than the local wild mosquitoes.
- The hypothesis regarding breakdown of effectiveness and selective mating. ***The suppression project described showed sustained suppression, even after releases of OX513A males stopped, as the authors of this paper themselves describe in an earlier publication (Garziera et al., 2017). Selective mating has never been observed in any releases of close to 1 billion Oxitec males worldwide. The authors provide no data to support this hypothesis***.

Unsubstantiated and inflammatory statements:

The paper includes a range of statements that are not substantiated by the study's data or by any other publication:

Article Title:

> ***"Transgenic Aedes aegypti Mosquitoes Transfer Genes into a Natural Population"***

The title is misleading and alarmist, making it sound as though OX513A is a gene-drive-type mosquito or that otherwise this phenomenon is unexpected or unknown. In contrast, the authors have themselves demonstrated the complete disappearance from the environment of the self-limiting and fluorescent genes in OX513A (Garziera et al., 2017). This should have been made clear in the title and in the abstract, as the self-limiting/lethal gene in OX513A does not remain in the wild population after releases have ended.

Abstract:

> ***"It is unclear how this may affect disease transmission or affect other efforts to control these dangerous vectors."***
>
> ***"These results highlight the importance of having in place a genetic monitoring program during such releases to detect un-anticipated outcomes."***

This is a meaningless statement, as it is not possible to design a monitoring program to detect unanticipated outcomes. By definition, monitoring can only take place to detect anticipated but potentially undesirable outcomes. Furthermore, the authors do not demonstrate any outcomes that might be cause for concern around either safety or efficacy of the OX513A strain, other than vector competence and insecticide resistance. The authors have demonstrated clearly and specifically that the OX513A strain has no increased vector competence relative to the local wild-type *Aedes aegypti*. A separate published study demonstrates that OX513A is susceptible to standard insecticides (for example, pyrethroids and organophosphates) used for mosquito control. Both studies confirm that the OX513A strain is a safe and appropriate choice. Finally, the above statement from the authors implies that Oxitec's mosquito releases are taking place without appropriate oversight: it is a matter of public record that, in Brazil Oxitec's commercial releases of OX513A are subject to a multi-faceted monitoring program, as legally required by the Brazilian biosafety regulatory CTNBio.

Introduction:

> ***"Release of this strain in large numbers has been effective in reducing populations of Ae. aegypti by up to 85%."***

The authors have selectively omitted references demonstrating that OX513A releases have been successful in reducing populations by up to 95% in Bahia, Brazil (Carvalho et al., 2015) and 93% in Panama (Gorman et al., 2016).

Discussion:

"However, it is clear from the data in Garziera et al (ref 6) that the effectiveness of the release program began to break down after about 18 months, i.e., the population which had been greatly suppressed rebounded to nearly pre-release levels."

The paper to which this statement refers (Garziera et al., 2017) shares four authors with the Scientific Reports paper in question, and those same authors make no such conclusion in this earlier paper describing the suppression program in Bahia state, Brazil. In fact, (Garziera et al., 2017) states that mosquito populations in the two treated areas remained suppressed for some time after OX513A released ceased: "*The mosquito population in Juazeiro (Mandacaru) remained suppressed for 17 weeks after the release interruption, whereas in Jacobina (Pedra Branca) suppression lasted 32 weeks.*" There is no evidence in (Garziera et al., 2017) to support speculation that the program started to break down while OX513A mosquito releases were under way. Again, this is a selective omission of a significant set of data with direct relevance to the paper's subject.

"This has been speculated to have been due to mating discrimination against OX513A males, a phenomenon known to occur in sterile male release programs (ref 16). This observation also implies that introgressed individuals may be at a selective disadvantage causing their apparent decrease after release ceased, although much more data would be needed to confirm this."

The 'speculation' referred to is from a paper by the corresponding author, Jeffrey Powell (Powell, 2018), and is therefore a self-citation. No evidence is presented to support this assertion, which is directly contradictory to the published data in the paper.

Furthermore, most sterile male release programs (whether insects are sterilised by irradiation, as for screwworm, fruit flies or other mosquitoes, or by genetics) have recorded no evidence of assortative mating (Alphey et al., 2010), which states "*Resistance through assortative mating has been reported in several cases, but was generally found to be associated with a loss of quality in the mass-reared insects, probably due to inbreeding; this was rapidly reversed by improved genetics. We know of only one instance of reasonably well-documented resistance through assortative mating* (McInnis, Lance & Jackson, 1996) *in the absence of clear decline of sterile insect production quality in the entire 50+ years of SIT.*"

"It is not known what impacts introgression from a transgenic strain of Ae. aegypti has on traits of importance to disease control and transmission."

This statement is inflammatory and dramatic with no basis, potentially used to inspire headlines or attention, or to degrade the technology's value in combating disease vectors. The authors have demonstrated clearly and specifically that the transgenic strain of *Aedes aegypti* used in this field trial, OX513A, has no increased vector competence relative to the local wild-type *Aedes aegypti*. The above speculative statement directly contradicts the authors' own findings, and speculates that OX513A (or its background genetics) may increase the potential for disease transmission by *Aedes aegypti*, without any basis for doing so, or without reference to the extensive body of literature that demonstrates that many of the factors most likely to affect vector competence are not genetic, but environmental, relating to the mosquito's microbiome and immune response, and relating to the genetics of the virus rather than the vector (Tabachnick, 2013; Palmer, Varghese & Van Rij, 2018; Souza-Neto, Powell & Bonizzoni, 2019). Secondly, the successful deployment of OX513A male

mosquitoes, which led to effective mosquito population reductions, is likely to reduce overall arbovirus transmission and break transmission cycles of arboviruses (Focks et al., 2000).

> ***"Also, introgression may introduce other relevant genes such as for insecticide resistance."***

The authors provide no scientific basis for this statement, which directly contradicts the published literature about the OX513A strain, which demonstrates that OX513A is susceptible to standard insecticides (for example, pyrethroids and organophosphates) used for mosquito control. The most significant and primary paper that describes such findings on OX513A (Carvalho et al., 2015) was co-authored by some of the authors of this article (M.L.C., A.M., L.G.), was also cited for other purposes in this article, and yet was ignored in relation to this point. Another omitted published article demonstrates the same results, i.e. that OX513A is susceptible to currently-used insecticidal chemistries (Patil et al., 2018).

Secondly, if any effect were to occur as a result of OX513A background genetics being introgressed into the local population, the effect would be expected to be beneficial, as introgression of insecticide-susceptible alleles would be expected to occur, restoring the effectiveness of insecticides against a local population that may have developed resistance. This was ignored by the authors.

> ***"The three populations forming the tri-hybrid population now in Jacobina (Cuba/Mexico/Brazil) are genetically quite distinct (Extended Data Fig. E2), very likely resulting in a more robust population than the pre-release population due to hybrid vigor."***

The authors provide no scientific basis for this statement, which is purely speculative, and which again contradicts their own data. Earlier in the Discussion they note that "*This observation also implies that introgressed individuals may be at a selective disadvantage causing their apparent decrease after release ceased*," hence demonstrating the opposite of 'hybrid vigor' in the context of fitness in the field. Again, this is an example of many within the paper wherein the editors missed some very clear factual inaccuracies relating to the paper and the very data it is presenting.

> ***"These results demonstrate the importance of having in place a genetic monitoring program during releases of transgenic organisms to detect un-anticipated consequences."***

This is a meaningless statement, as it is not possible to design a monitoring program to detect unanticipated outcomes. By definition, monitoring can only take place to detect anticipated but potentially undesirable outcomes. Furthermore, the authors do not demonstrate any outcomes that might be cause for concern around either safety or efficacy of the OX513A strain, other than vector competence and insecticide resistance. In this paper, the authors have demonstrated clearly and specifically that the OX513A strain has no increased vector competence relative to the local wild-type *Aedes aegypti*. A separate published study demonstrates that OX513A is susceptible to standard insecticides (for example, pyrethroids and organophosphates) used for mosquito control. Both studies confirm that the OX513A strain is a safe and appropriate choice (Carvalho et al., 2015). Finally, the above statement from the authors implies that Oxitec's mosquito releases are taking place without appropriate oversight: it is a matter of public record that, in Brazil Oxitec's commercial releases of OX513A are subject to a multi-faceted monitoring program, as legally required by the Brazilian biosafety regulatory CTNBio.

Oxitec's Self-Limiting Aedes aegypti OX5034 is not a Gene Drive Technology

Oxitec does not use gene drive in its self-limiting insects. Unlike gene drive technologies, Oxitec's technology is self-limiting, which means Oxitec insects cannot establish in the wild.

Key Facts

- Oxitec's self-limiting technology works in the opposite way from gene drive. Oxitec's 2nd Generation mosquitoes carry two copies of the self-limiting gene. When Oxitec's males mate with wild females, the self-limiting gene persists only in males. Females that inherit the gene cannot survive to reproduce. Therefore, the self-limiting gene gradually declines in the population gene pool and cannot persist, enabling potential population suppression across multiple generations before the gene is eliminated from the environment.

- By releasing enough self-limiting male insects over a sustained period to mate with pest females and thereby reducing the number of female progeny, the pest population is suppressed. In contrast to the design of gene drive technologies, if releases of Oxitec males cease, the pest population can recover. As female carriers of the self-limiting gene cannot survive to reproduce, the self-limiting gene also cannot establish or become invasive in the wild.

- Introgression into the wild mosquito population of natural mosquito genes present in OX5034 is expected to occur, and these genes are also expected to disappear from the environment over time. The natural background genetics of OX5034 were selected to ensure that OX5034 is susceptible to commonly-used insecticides, meaning that introgression of these genes into the wild population has the potential to help make the wild population less resistant to insecticides used to control mosquitoes.

- Gene drive is a genetic engineering technology that propagates genes throughout a population without any off-switch. As a result, the gene drive insertion in the genome will re-occur in each individual insect that inherits one copy of the modification and one copy of the wild-type gene. The gene drive gene is thereby designed to convert wild-type (unmodified) counterparts into gene drive too. Therefore, these systems are designed to eventually become established or fixed in the population. Gene drive thus spreads and persists in the environment.

Tetracycline Usage and Potential Antibiotic Resistance

The potential for Oxitec's mosquito technology and its subsequent deployment to lead to increased risk of antibiotic resistance, is negligible. Oxitec makes use of a small level of tetracycline-family antibiotics in the rearing of our 2nd generation mosquito eggs in its facility in the UK. Oxitec technology does not increase risk of antibiotic resistant bacteria in the environment where egg manufacture or releases are carried out (as confirmed by the FDA in 2016[1]), and Oxitec will not be using any tetracycline or any other antibiotic in the US.

Key Facts

- **No tetracycline or other antibiotics will be used in rearing of Oxitec's non-biting male mosquitoes for the pilot project in the Florida Keys; no tetracycline will be released into the environment in the US; Oxitec will have no tetracycline in the US.**
- When the FDA approved Oxitec's 1st generation technology in 2016[1], it considered Oxitec's use of antibiotics and determined that there is no risk to humans, animals or the environment from use in Oxitec's rearing processes in the US. Now Oxitec's 2nd generation technology does not use any tetracycline/doxycycline in the US for rearing of male mosquitoes for release into the environment.
- **The Oxitec male OX5034 mosquitoes reared for release in Florida will never have been in contact with tetracycline, and therefore the risk of spreading tetracycline-resistant bacteria is negligible.**
- Oxitec will use only a small level of doxycycline, a common, widely-used member of the tetracycline family, only in the UK, to rear females which will not be released (but which produce the mosquito eggs to be used in Florida).
- The amount of doxycycline that would be used in the UK to produce the females that would supply all the eggs needed for the EUP is less than 5 grams.
- For comparison, this is about the equivalent of two 10-day courses of antibiotics to treat a normal infection. More than 6.5 million courses of doxycycline were prescribed in the US in 2016[2].
- In addition, the EPA[3], federal and Florida state governments have approved the deployment of hundreds of tons of antibiotics into Florida's environment annually for agricultural and food production purposes[4].
- Agricultural use of antibiotics in Florida alone is 88 million times more than what Oxitec will use in the UK.
- Therefore, existing human and agricultural uses of tetracyclines are far more likely to result in the spread of antibiotic-resistant bacteria, than Oxitec's very limited use of doxycycline outside of the USA.
- **Environmental levels of tetracyclines high enough to help female OX5034 mosquitoes survive have never been recorded in the USA in potential *Aedes aegypti* breeding sites**, based on a comprehensive survey of the peer-reviewed literature (Meyer et al., 2000; Lindsey, Meyer & Thurman, 2001; Campagnolo et al., 2002; Yang & Carlson, 2003; Yang, Cha & Carlson, 2004, 2005; Kim et al., 2005; Karthikeyan & Meyer, 2006; Batt, Bruce & Aga, 2006; MacKie et al., 2006; Batt, Kim & Aga, 2007; Dolliver & Gupta, 2008b,a; Haggard & Bartsch, 2009; Kulkarni et al., 2017). The highest reported concentrations

[1] https://www.fda.gov/files/animal%20&%20veterinary/published/Oxitec-Mosquito---Finding-of-No-Significant-Impact.pdf

[2] https://clincalc.com/DrugStats/Drugs/Doxycycline

[3] EPA, Final Registration Decision for the New Use of the Active Ingredient Oxytetracycline Hydrochloride on Citrus Crop Group 10-10 (Dec. 7, 2018), www.regulations.gov/document?D=EPA-HQ-OPP-2015-0820-0031

[4] https://www.nature.com/articles/d41586-019-00878-4

of environmental tetracyclines would be insufficient to allow survival of any female hemizygous OX5034 life-stages. The testing of antibiotic concentrations found in the environment is frequently associated with the efficacy of waste water treatment plants at removing antibiotics from waste water. Samples are taken from influent and effluent, and from rivers downstream of treatment plants. Antibiotic concentrations are also frequently tested in hog lagoons, which are anaerobic lagoons used to treat animal waste from farming pigs or other livestock. These are not typical breeding locations for *Ae. aegypti* larvae. *Ae. aegypti* is commonly referred to as a 'container breeding mosquito' as its preferred breeding sites include flower vases, tires, tree holes, etc. They are found in clean, still water, not flowing river systems and are rarely found in collections of water in the ground such as borrow-pits or earth drains (Christophers, 1960; Morrison et al., 2006; Dieng et al., 2012). Some reports have suggested that *Ae. aegypti* can breed in septic tanks (Barrera et al., 2008; Mackay et al., 2009) but this tends to be in the clear water at the top of the tank whereas tetracycline and their analogues tend to bind to the sediment which collects at the bottom (Brown et al., 2006; Watkinson et al., 2009). Therefore, the concentrations that resulted in functional female phenotypic rescue in this study are very unlikely to be found in typical breeding sites of *Ae. aegypti* (Curtis et al., 2015), therefore the potential for the efficacy of a control program using OX5034 to be compromised by the current reported levels of environmental tetracycline and its analogues is negligible.

Thank you again for the opportunity to provide comments related to this EUP application.

Sincerely,

Nathan Rose, DPhil

Head of Regulatory Science

Oxitec Ltd

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